REMARKS

Applicants thank Examiner Srivastava and Examiner Naff for the courtesies extended during a personal interview on March 18, 2008. The claim amendments set forth above, and the comments below, reflect the matters discussed at that interview.

Claims 5-8, 13, and 18-39 have been canceled, claims 1-3 and 17 have been amended and claims 40-41 have been added. The claims amendments and added claims are fully supported by the specification and original claims.

In the February 25, 2008, Office Action, claim 1-17 were rejected under 35 USC § 102(b) as anticipated by Wang, and claims 1-4 and 7-17 were rejected under § 102(b) as anticipated by Banerjee. The specific grounds for rejection, and applicants' response thereto, are set forth in detail below.

Support for amendments

The amendments to claim 1 are supported in the abstract, original claims 5-18 and 13, and in the specification at page 2, line 10, page 3, lines 9-20, page 5, lines 3-6, page 8, lines 14-20, page 10, line 17, and Figures 1 and 2. The remaining amendments are ministerial in nature.

Rejections Under 35 U.S.C. §102(b)

Claims 1-17 are rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,672,696 by Wang et al. ("Wang"). Claims 1-4 and 7-17 are rejected under 35 U.S.C. § 102(b) as being anticipated by Banerjee et al. ("Banerjee")(BioTechniques vol. 18:768-73, 1995). Applicants respectfully traverse.

It is axiomatic that, for a prior art reference to be anticipatory, every element of the claimed invention must be identically shown in a single reference. *In re Bond*, 15 USPQ2d 1566 (Fed. Cir. 1990). Neither Wang nor Banerjee describes every element of the claimed invention and, accordingly, neither reference can anticipate the claimed invention.

The pending claims recite a protease digestion step that uses a protease selected from the group consisting of trypsin, chymotrypsin, and endoproteinase Lys-C. By contrast, Wang describes a protease treatment that uses papain, whereas Banerjee uses proteinase K. Neither of the cited references describes use of the proteases recited in claim 1. Accordingly, neither

reference teaches each and every element of the claimed invention and applicants respectfully request withdrawal of the rejection.

Moreover, applicants respectfully point out that neither Wang nor Banerjee renders obvious the instantly claimed invention. Thus, the instantly claimed invention is directed to methods of preparing a lysate from a biological sample where, surprisingly, the protein content of the lysate is representative of the total protein content of the original biological sample and is present in a soluble form that is suitable for analysis. By contrast, both Wang and Banerjee are directed to methods of preparing lysates where the *nucleic acid* component of the lysate is of primary interest and where any protein or peptide content of the lysate is essentially an unwanted contaminant. Neither Wang nor Banerjee teach or suggest that a lysate can be prepared that contains protein and protein fragments that are representative of the protein content of the sample from which the lysate is prepared – indeed, neither Wang nor Banerjee suggest that such as lysate would be either desirable or useful, let alone possible. In addition, the protein and peptide content of the lysates obtained by Wang and Banerjee is very low and is not remotely representative of the original protein content of the original biological sample, as described in more detail below.

As an initial matter, both Wang and Banerjee use heat treatment steps of a nature such that the following protease cleavage does not, and likely cannot, create a soluble lysate. Rather, both Wang and Banerjee's methods produce soluble and insoluble fractions, where the majority of the protein content appears to be in the insoluble fraction (see below). Moreover, both Wang and Banerjee use protease cleavage steps where the protease cleaves promiscuously, in contrast to the instantly claimed invention, where the proteases recited in the claims cleave proteins at specific sites and in limited fashion.

In this sense, both Wang and Banerjee may be understood as teaching away from the instant invention: whereas the instantly claimed methods produce lysates that are rich in protein information, both Wang and Banerjee want to remove as much protein as possible, since it is the nucleic acid that is of interest and the protein is essentially a contaminant.

Applicants' prior submission filed October 31, 2007, contained a declaration pursuant to 37 CFR § 1.132 by Marlene Darfler (the "Darfler Declaration"), which compared the methods of the instant application as recited in claim 1 and the methods described by Wang and Banerjee. Ms. Darfler determined that the protocols disclosed by Wang and Banerjee do not result in a

biomolecule lysate in a soluble liquid form suitable for protein analysis, and that the biomolecule lysate is not representative of the total protein content, as required by claim 1 of the instant application. (Darfler Declaration at ¶¶ 10, 19 and 24.)

Formalin fixed paraffin embedded mouse liver tissue was used as the starting material in each protocol. (Darfler Declaration at ¶10.) Ms. Darfler indicates that using Expression Pathology's Liquid Tissue MS Protein Prep Kit manual is consistent with the method of claim 1, and results in a biomolecule lysate contained in a single tube that is in a visibly soluble liquid form.

In contrast, performing the Wang protocol as provided in Example 1 of Wang results in three separate fractions: an insoluble fraction, a visibly soluble fraction, and a DNA fraction rendered visibly soluble after DNA precipitation. (Darfler Declaration at ¶11.) The Liquid Tissue lysate and the two visibly soluble fractions from the Wang protocol were subjected to mass spectrometric (MS) analysis, which is capable of identifying thousands of individual peptides and proteins in a single analysis and thereby providing an overall representation of protein expression in a biomolecule lysate. (Darfler Declaration at ¶13.) The Liquid Tissue protocol identified 1,251 different, unique proteins from a sample of formalin fixed paraffin embedded mouse liver tissue. (Darfler Declaration at ¶15.) In contrast, only 107 different, unique proteins were identified in the liquid fraction resulting from the Wang protocol, and only 12 different, unique proteins were identified in the resuspended DNA fraction from the Wang protocol. (Darfler Declaration at ¶¶ 16-17.) Similarly, soluble fraction of the Banerjee lysate contained only 15 identifiable proteins.(Darfler Declaration at ¶¶ 15, 18.)

Ms. Darfler also describes how analyses were performed using the Gene Ontology (GO) program to identify the representation of various sub-cellular locations in a given biomolecule lysate. (Darfler Declaration at ¶20.) While GO analysis of the Liquid Tissue lysate demonstrated the isolation of proteins from 124 different regions across every part of the cellular milieu, GO analysis of the Wang liquid fraction returned only 30 regions, and the proteins resulting from the DNA fraction of Wang were from only 8 different regions. (Id.) The Banerjee lysate provided proteins from only 8 regions. (Id).

Further, the relative content of proteins involved in liver function was determined among the Liquid Tissue preparation biomolecule lysate and the fractions obtained using the Wang and Banerjee protocols. (Darfler Declaration at ¶21.) In the Liquid Tissue lysate, a total of 677

proteins of the 1,251 proteins identified are involved in normal liver function, while there were 10 liver function proteins identified in the Wang liquid fraction lysate and 3 in the Wang DNA fraction lysate. (Id.)

Finally, Ms. Darfler notes that in the experimental analyses performed under her direction, the Liquid Tissue lysate contained all four of the standard proteins that are produced by the liver and whose presence is assayed for in the blood in a widely-applied clinical assay: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase, and albumin. (Darfler Declaration at ¶22.) None of these four proteins were detected in either of the Wang fractions nor in the Banerjee lysate. (Id.)

On the bases of these empirical results, Ms. Darfler concludes that the Liquid Tissue lysate is representative of the starting material originating from a histopathologically processed liver sample. (Darfler Declaration at ¶23.) Furthermore, it is Ms. Darfler's opinion that the lack of liver function proteins identified in the Wang and Banerjee preparations indicates that these lysates are not representative of the total protein content of the starting material originating from a histopathologically processed liver sample. (Darfler Declaration at ¶¶23-24.)

In summary, for the reasons set forth above, neither Wang nor Banerjee anticipate the instantly claimed invention. In addition, neither Wang nor Banerjee teach or suggest either the desirability or possibility of preparing a lysate from chemically fixed tissue where the peptide and protein content of the lysate is representative of the protein content of the sample from which the lysate was prepared. Moreover, for the reasons set forth above and in the Darfler declaration, the methods described by Wang and Banerjee fail to provide a liquid, soluble, dilutable biomolecule lysate that is suitable for protein analysis and where the protein content of the lysate is representative of the total protein content of the sample from which the lysate was prepared. Accordingly, withdrawal of the rejection respectfully is requested.

CONCLUSION

In view of the foregoing amendments and remarks, applicants respectfully submit that the application is in condition for allowance. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact the undersigned to expedite prosecution of the application.

The Commissioner is hereby authorized by this paper to charge any fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-3840. **This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).**

Respectfully submitted,

Date: April 9, 2008

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